REMARKS

I. Introduction.

Claims 1-32 were examined. Claims 33-43 have been canceled, as drawn to the nonelected invention. The cancellation of claims is done without prejudice to further prosecution in a divisional application.

Claims 1-25, 27-32 and 44 are pending. Claims 1, 14, 19, 20, 21, 24, 25, 29, 30, and 32 are amended. These claims are amended to more specifically claim certain embodiments of the instant invention. The invention, as now more specifically claimed with the amendments, is directed to targeting activated MLK activity in neuronal cells with a compound to determine that compound's ability to prevent neuronal cell death. The amendments of the claims are done without prejudice to further prosecution of other embodiments of this invention in a continuation, continuation-in-part, divisional, or other related application.

This invention is based on the discovery that expression of MLK activates the SEK1-JNK pathway and induced apoptosis in neuronal cells. Thus, over-activation or stimulation of the MLK-SEK1-JNK cascade mediates neuronal toxicity. Since over-activation of glutamate receptor induces excitotoxicity in neurons and is a common pathway responsible for neuronal death in a variety of neurodegenerative diseases and in various acute insults such as hypoxia, ischemia, stroke, and others, the over-activation of the MLK-SEK1-JNK pathway serves as a common molecular mechanism for neuronal loss in these illnesses. Therefore, inhibition of the activation of the MLK-SEK1-JNK pathway by suppressing MLK-associated activities will prevent neuronal death in these neurological diseases. Prior to Applicant's invention, there was no report on the role of MLK activation in any kind of neuronal toxicity.

II. Priority Application

The Examiner states that Applicant is given the benefit of the filing date of September 17, 1998, and is being denied benefit to the provisional application filing date of May 14, 1998. According to the Examiner the basis for this decision is that "the full scope embraced by each claim was not disclosed in the provisional application."

Applicant respectfully submit that she is entitled to priority to the May 14, 1998, date for as much as what is disclosed and fully supported by the provisional application.

The priority application provides support for a method of screening or assessing a compound's ability to prevent neuronal cell death by containing a compound with neuronal cells having both activated JNK or MLK activity. The invention, as now claimed, is directed to MLK activity. The Examiner's attention is directed to the provisional patent application at the following pages which provide support for MLK activity:

Page 2, lines 8-15:

Moreover, normal huntingtin is associated with MLK2, a nuclear kinase which is almost exclusively expressed in brains. Expression of MLK2 also activated the SEK1-JNK pathway and induced apoptosis in HN33 cells. Co-expression of mutated huntingtin with MLK2 induced apoptosis in 293 cells while expression of normal or mutated huntingtin or MLK2 alone, or co-expression of normal huntingtin with MLK2 did not generate any toxic effect. Taken together, our studies demonstrate that expression of polyglutamine-expanded huntingtin induces neuronal apoptosis by activation of the SEK1-JNK pathway and this effect may be mediated by MLK2.

Page 5, last paragraph to page 7, line 16:

We then investigated the signal transduction pathway involved in activation of the SEK1-JNK pathway. In a previous study, we demonstrated that huntingtin interacts with SH3 domain-containing proteins such as Grb2 and RasGAP (18).

Members of the MLK family are the only known SH3 domain-containing proteins which activate JNKs (19-21). MLKs directly bind and activates [*sic* activate] SEK1 which in turn elevates the JNK activity. Since MLK2 is a neuronal form of MLKs, we examined the effect of MLK2 expression in HN33 cells. NO any [*sic* No] toxic effect was observed in HN33 cells transfected with pRK5CMV vector (Fig. 4A, control). Expression of MLK2 caused apoptotic cell death in HN33 cells and co-expression of dominant negative SEK1 mutant (SE1K/R) attenuated MLK2-mediated neuronal apoptosis (Fig. 4A). Dominant negative SEK1 was clearly more effective at inhibiting cell toxicity induced by mutated huntingtin than by MLK2. This observation is consistent with other reports (20-21) and reflects the fact that MLK2 is a constitutively active kinase whose action is more difficult to block although SEK1 is direct downstream of MLK2. Mutated huntingtin, on the other hand, lacks enzyme activity and may regulate activity of endogenous MLK2 in HN33 cells. Therefore, its action on the SEK2-JNK pathway may be more easily attenuated. Taken together, these results demonstrate that MLK2, like mutated huntingtin, also activates the SEK1-JNK pathway to induce cell death in HN33 cells.

Next, we explored the potential association of huntingtin with MLK2. Because expression of either mutated huntingtin or MLK2 alone cause rapid cell death in HN33 cells (Table 1), we could not obtain sufficient cells to perform a co-immunoprecipitation experiment. No toxic effect was observed in 293 cells transfected or co-transfected with control vectors (Table 1). Expression of MLK2, or normal or polyglutamine-expanded huntingtin alone in 293 cells also did not generate cell toxicity at 48 hours after transfection (Table 1) and expression of normal or mutated huntingtin failed to activate JNKs (data not shown). Since 293 cells are rich in huntingtin (18), we examined the interaction of MLK2 with normal huntingtin in 293 cells. c-Myc tagged MLK2 was transiently expressed in 293 cells and MLK2 was precipitated with anti-c-myc tag 9E10 antibody (22). In cell lysates from 293 cells transfected without (Fig. 4B, 9E10IPC) or with pRk5CMV vector alone (Fig. 4B, 9E10IPV), 9E10 failed to co-precipitate huntingtin (Fig. 4B), while huntingtin was easily detected in both 9E10 (Fig. 4B, 9E10IPT) and anti-huntingtin (Fig. 4B, HDPIP) immunoprecipitates of cell lysates from 293 cells transfected with c-myc tagged MLK2. Conversely, we determined whether an anti-huntingtin antibody precipitates MLK2. Cell lysates from 293 cells with or without transfection of MLK2 were incubated with 437, an anti-huntingtin antibody or 9E10. In non-transfected wild-type 293 cells, MLK2 was not detectable in both 9E10 and 437 immunoprecipitates (Fig. 4C, 9E10IPC) and in pRK5CMV transfected cells, MLK2 was also absent in 9E10 immunoprecipitates (Fig. 4C, 9E10IPV). Whereas in MLK2 transfected 293 cells, MLK2 was detected in both 9E10 and 437 immunoprecipitates (Fig. 4C, 9E10IPT

& 437IPT). Since the detected association of MLK2 with endogenous huntingtin only takes place in MLK2 transfected 293 cells, not in wild-type 293 cells or 293 cells transfected with pRK5CMV expression a c-myc tag alone, these studies suggest that normal huntingtin interacts with MLK2 in intact cells. Next we attempted to examine a potential difference between normal and mutated huntingtin interaction with MLK2. Co-transfection of pFL16HD, which expresses normal huntingtin with a 16 CAG repeats, with MLK2 vector did not produce any cell toxicity (Table 1). Although expression of mutated huntingtin or MLK2 alone did not influence 293 cell vitality (Table 1), co-expression of mutated huntingtin with MLK2 induced rapid apoptosis and most cells died within 48 hours after transfection (Table 1). Thus, we could not obtain enough 293 cells co-transfected with MLK2 and mutated huntingtin to perform [a] co-immunoprecipitation experiment to determine any alteration of the interaction of MLK2 with polyglutamine repeat-expanded huntingtin. However, these results do suggest that MLK2 and mutated huntingtin synergistically stimulate the apoptosis signaling pathway in 293 cells."

Page 8, last paragraph to Page 9, line 16:

Several observations in our study suggest that MLK2 may be in involved mutated huntingtin-induced neuronal apoptosis. First, MLK2, like mutated huntingtin, initiates apoptotic cell death in HN33 cells. Second, mutated huntingtin, like MLK2, induces JNK activation. Third, neuronal toxicity induced by either mutated huntingtin or by MLK2 could be attenuated by dominant negative SEK1 which specifically inhibits JNK activation. Fourth, co-expression of normal huntingtin with MLK2 does not induce apoptosis in 293 cells while co-expression of mutated huntingtin with MLK2 results in cell death. Furthermore, MLK2 is present in HN33 cells and absent in 293 cells according to a RT-PCR analysis (data not shown). Thus, mutated huntingtin-mediated cell toxicity requires the presence of MLK2. The precise mechanism for how MLK2 is directly involved in mutated huntingtin-mediated neuronal apoptosis is not clear. Since normal huntingtin is associated with MLK2 in intact cells and such an association does not generate any cell toxicity, it may be possible that normal huntingtin is an inhibitor of MLK2 while expansion of the polyglutamine repeat in huntingtin interferes with its association with MLK2 and leads to activation of the SEK1-JNK pathway. As a result, mutated huntingtin may act as a dominant negative mutant and attenuate the inhibitory action of normal huntingtin on the MLK2-SEK1-JNK pathway. This may explain why HD is inherited in a dominant fashion. Currently, we are further investigating how MLK2 is involved in mutated huntingtin-mediated neuronal toxicity. In addition to huntingtin, most

proteins involved in CAG-expanded hereditary diseases, such as ataxin 1, ataxin 2, ataxin 6, ataxin 7, DRPLA, and androgen receptor (27), all contain one or more potential SH3 domain binding motifs and potentially , they may all bind to MLK2. Thus, activation of the MLK2-SEK1-JNK pathway may be one of common molecular mechanisms for neuronal loss in CAG-expansed neurodegenerative diseases.

The Examiner's attention is also directed to Figure 4 and Table 1 of the provisional application which provides enabling support as required under 35 U.S.C. § 112, first paragraph, for the claims of the instant application.

The instant application is admittedly an application that contains additional information to that of the provisional application. Applicant submits that the provisional application fully supports and enables the instant application. Further, this situation is analogous to that of filing a continuation-in-part application from an earlier filed application. As stated in MPEP ¶ 706.02, page 700-10, any claims of the cip application which are fully supported under § 112 by the earlier parent application will have the effective filing date of that earlier parent application.

III. Rejection Under Section 102(a) based on Liu et al., Society for Neuroscience Abstracts, October 1997.

Claims 1-2, 4, 6, 8-9, 11, 13-15, and 17 are rejected under 35 U.S.C. § 102(a) as anticipated by Liu et al., Society for Neuroscience Abstracts, October, 1997. According to the Examiner, Liu et al is cited as it is authored by other people in addition to the named inventor. In addition, the Examiner states that "Liu et al. teaches that expression of the huntingtin mutant activates JNK/APK and induces neuronal apoptosis in hippocampal cells. Dominant-negative SEK(K-r) inhibits this induced apoptosis and may be a therapeutic tool in Huntingtin's Disease."

Applicant is submitting with this Amendment an *In re Katz* type declaration under 37 C.F.R. §1.132. With the filing of this declaration, this rejection should now be moot.

III. Rejection Under Section 103(a) based on Liu et al., Society for Neuroscience Abstracts, October 1997.

Claims 9-10, 14, and 18 are rejected under 35 U.S.C. § 103(a) as obvious over Liu et al., Society for Neuroscience Abstracts, October, 1997.

With the filing of the *In re Katz* declaration under Rule 132, it is submitted that this rejection is now moot.

IV. Rejection Under Section 103(a) based on Yardin, et al, Neuroreport, (June 22, 1998), Ni et al, U.S. 5,840,509, and Johnson, U.S. 5,854,043.

Claims 1-2, 5-9, 12-16, and 19-32 are rejected under 35 U.S.C. § 103(a) as obvious over the combination of Yardin, et al, Neuroreport, June 22, 1998, Ni et al, U.S. 5,840,509, and Johnson, U.S. 5,854,043. According to the Examiner, "It would have been obvious to use the experimental system of Yardin et al. to assess a compound's ability to prevent neuronal cell death in neurological conditions. . . . Johnson, Yardin et al., and Ni et al. make clear that it would have been well known how to manipulate various aspects of the second messenger systems to evaluate inhibitors and induces of apoptosis, enzymatic activity, gene expression, and so forth in the cascade, using well known techniques."

Applicant respectfully traverses this rejection.

To begin, Yardin et al. is a reference with a date as of June 22, 1998. Applicant is entitled to a priority date of May 14, 1998. For that reason, Yardin et al. is not an appropriate reference.

Even if Yardin et al. were an appropriate reference, Yardin et al. does not render obvious the invention, as now claimed, obvious.

Yardin et al. demonstrate that FK506, an immunosuuppressive drug, can block neuronal apoptosis induced by serum deprived cortical (neuronal) cell cultures. The results showed that the FK506 prevented the expression of the C-jun protein in these serum deprived (stressed) neuronal cell cultures. Yardin et al. state that "The links between the association of FK506-FKBP12 and C-jun are not known but could implicate ATF-2 in the induction of C-jun transcription. Further work will be needed to analyze the relationship between C-jun, ATF-2 and FK506 since ATF-2 [activating transcription factor-2] is also a substrate for the C-jun kinases which phosphorylate C-jun protein." (Yardin et al, page 2080, last paragraph.)

c-Jun is a transcription factor and its expression can be influenced or regulated by a number of factors, including JNK phosphorylation and in some cell types by ERK-mediated mechanisms. (See the review article, Leppa and Bohmann, "Diverse functions of JNK signaling and c-Jun in stress response and apoptosis," *Oncogene* 18:6158-6162 (1999)and Leppo et al., "Differential regulation of c-Jun by ERK and JNK during PC12 cell differentiation," *EMBO Journal* 17:4404-4413 (1998). Copies of these articles are enclosed for the convenience of the Examiner.) Thus, as Yardin et al. demonstrate, other factors influence the expression of c-Jun.

Yardin et al. does not recognize, much less render obvious, Applicants invention as now claimed, namely, that the inhibition of MLK activities in the MLK-SEK1-JNK pathway will prevent neuronal death in these neurological diseases.

Ni et al. is directed to a neuronal interleukin-1 converting enzyme (ICE) related protease and related DNA compounds and the use of this protease and its DNA in a method to identify compounds that inhibit the apoptotic process. Ni et al. states that

"ICE related protease is present in the CNS and is enriched in central neurons including pyramidal neurons and granule neurons of the hippocampus and cerebral cortex. Data suggests that overexpression of ICE related protease could be involved in a neuronal death cascade in mammalian neurons. A further understanding of the cellular events underlying apoptosis will probe useful for developing neuroprotective strategies as well as therapeutic interventions for head traumas, ALS, Alzheimer's stroke, brain ischemia, as well as a variety of other neurodegenerative disorders involving apoptosis." Col. 7, lines 43-53.

Applicant agree with the Examiner that it was known "how to manipulate various aspects of the second messenger systems to evaluate inhibitors and induces of apoptosis, enzymatic activity, gene expression, and so forth in the cascade, using well known techniques." Applicants disagree, however, that the combination of references teaches one of skill in the art that MLK can be used as a target for the development of inhibitory compounds of MLK-associated activity.

Johnson teaches a method for regulating the homeostasis of a cell by regulating the signal transduction activity of a mitogen extracellular signal-regulated kinase (ERK) kinase kinase protein (MEKK)-dependent pathway. Johnson discusses various ways in which the homeostasis of a cell is controlled by regulating the activity of an MEKK-dependent pathway in which the MEKK protein regulates the pathway substantially independent of Raf. Johnson also discusses regulating MEKK-dependent pathway by "contacting a cell with a compound capable of directly interacting with a protein including MEKK, JEK, JNK, Jun, ATF-2, and Myc, and combinations thereof, in such a manner that the proteins are activated; and/or contacting a cell with a compound capable of directly interacting with a protein including Raf, MEK, MAPK, TCF protein and combinations thereof in such a manner that the activity of the proteins are inhibited."

While Johnson does discuss increasing or decreasing the activity of JNK or Jun, it does not suggest to one of skill in the art that MLK can be used as a target for the development of inhibitory compounds of MLK-associated activity, and that such compounds can be used to prevent neuronal loss.

The combination of the cited references, Yardin et al., Ni et al., and Johnson does not render obvious Applicant's invention as now claimed. The claims, as now amended, are specifically directed to assessing or screening for a compound's ability to prevent neuronal cell death by contacting a compound with neuronal cells having activated MLK activity and determining by comparison the compound's ability to prevent cell death by the number of neuronal cells that die.

V. ***Rejection Under Section 103(a) based on Cheung et al, Journal of Neuroscience Research, (April 1, 1998), Ni et al, U.S. 5,840,509, and Johnson, U.S. 5,854,043.***

Claims 1-2, 5-7, 9, 12, 14-16, 19-22, and 24-32 are rejected under 35 U.S.C. § 103(a) as obvious over the combination of Cheung et al, Journal of Neuroscience Research, (April 1, 1998), Ni et al, U.S. 5,840,509, and Johnson, U.S. 5,854,043. According to the Examiner, "it would have been obvious to use the experimental system of Cheung et al. to assess a compound's ability to prevent neuronal cell death in neurological conditions. Apoptosis would have been well known to be involved in neurological conditions and the prior art teaches the association between c-Jun and apoptosis. Johnson, Cheung et al., and Ni et al. make clear that it would have been well known how to manipulate various aspects of the second messenger systems to evaluate inhibitors and induces of apoptosis, enzymatic activity, gene expression, and so forth in the cascade, using well known techniques."

Applicant respectfully traverses this rejection.

Cheung et al. studied the relationship between the expression of c-Jun and kainate-induced cell death in cerebellar granule cells. Cheung et al. states that kainate-induced cell death correlated with an increase in c-Jun mRNA and suggest that c-Jun expression is activated by apoptotic stimuli and is a potential marker for apoptosis. (See Cheung et al., page 78, second column, first paragraph.)

However, Cheung et al. does not teach nor suggest to one of skill in the art that MLK can be used as a target for the development of inhibitory compounds of MLK-associated activity. Even with the combination of Ni et al. and Johnson et al., one of skill in the art would only be left with the general suggestion of manipulating cellular pathways to evaluate inhibitors of apoptosis. This general suggestion is an invitation to invention, and does not render the claimed invention obvious.

VI. Conclusion.

Applicants respectfully submit that all the basis for rejection of the pending claims are now moot. The Examiner is requested to reconsider the rejections and to withdraw them and to pass this case to issuance.

Respectfully submitted,

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